

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

App. No. : 10/699,511 Confirmation No. 3571  
Applicant : Bennett, George N.  
Filed : October 31, 2003  
TC/A.U. : 1637  
Examiner : Calamita, H.  
Docket No. : 31175413-002002  
Customer No. : 51738  
Entitled : Method for assembling PCR fragments of DNA

MS Amendment  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION OF GEORGE N. BENNETT UNDER 37 CFR §1.132**

I, George N. Bennett, Declare as follows:

I am at least 18 years of age and am competent in all respects to make the following statements.

I am a joint inventor for claims 1-7 currently pending in US Patent Application No. 10/699,511.

I have read and understand the above-referenced application and pending claims.

I am a person of ordinary skill in the art of assembling nucleic acids including assembling PCR fragments with recombinases, see the attached *curriculum vitae*.

The present invention is the **first** demonstration of removal and circularization of a previously linear DNA from a solid support using a recombinase. *Cre/lox* recombination is affected by three factors: the method of release from the solid support, the length of the DNA to be recombined and relative concentrations of both the DNA and active Cre enzyme. Prior to this invention, it was not known if the Cre enzyme would release the DNA from solid support or if the DNA would be able to undergo further processing. It was not known if length of DNA attached to the solid support would affect Cre activity. Finally, the relative concentration of Cre

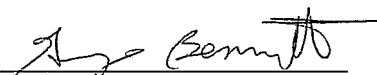
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enzyme required for activity under these conditions was unknown. Therefore it was not known if the Cre enzyme would work efficiently with a linear DNA substrate attached to a solid support.

Prior to the present invention, the ability of the CRE protein to function on immobilized DNA was unknown. The use of immobilized DNA for the *Cre/lox* recombination was not thought possible because immobilized DNA has a different topological structure than either native DNA *in vivo* or purified DNA *in vitro*. The *Cre/lox* reaction changes the topological structure of the DNA substrate. A DNA structure tethered to a solid support might not undergo the conformational changes required for recombination. One of ordinary skill in the art would have thought *Cre/lox* recombination was inhibited or impossible on a solid support.

I further declare that all statements made herein of my own knowledge are true and made on information believed to be true; further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of any application for which it is used.

Dated: May 8 2007

By   
George N. Bennett  
Dept. of Biochemistry and Cell Biology  
Rice University  
P.O. Box 1892 MS 140  
Houston, TX 77251

## **GEORGE N. BENNETT**

**TITLE:** Chair and E. Dell Butcher Professor of Biochemistry and Cell Biology

**EDUCATION:** University of Nebraska, Lincoln, Nebraska, B.S., 1968 (Chemistry)  
Purdue University, West Lafayette, Indiana, Ph.D., 1974 (Biochemistry)  
Stanford University, Palo Alto, California, Postdoctoral, 1975 - 1978

### **RESEARCH AND PROFESSIONAL EXPERIENCE:**

1. Chair, Biochemistry & Cell Biology, Rice University, 2003-present.
2. Professor of Biochemistry, Rice University, Houston, Texas, 1992-present.
3. Associate Professor of Biochemistry, Rice University, Houston, Texas, 1984-1992.
4. Assistant Professor of Biochemistry, Rice University, Houston, Texas, 1978-1984.
5. Postdoctoral Research Fellow, Department of Biological Sciences, Stanford University, 1975-1978.
6. Graduate Student, Department of Biological Sciences, Purdue University, 1968-1969 and 1971-1974.
7. Laboratory Technician, military service, Medical Biochemistry Laboratory, LAIR, Presidio, San Francisco, California, 1969-1971.
8. Research Assistant, USDA, Northern Regional Research Laboratory, Peoria, Illinois, 1968.

### **HONORS and RECOGNITION:**

University of Nebraska Foundation Scholarship  
University of Nebraska Regents Scholarship  
Phi Lambda Upsilon-Merck Award  
Pi Mu Epsilon  
Phi Eta Sigma  
European Molecular Biology Organization Conference Fellowship (1973)  
National Institutes of Health Postdoctoral Research Fellowship (Stanford University, 1975-1978)  
Elected Member of American Society of Biological Chemists (1981)  
American Men and Women of Science  
Outstanding Faculty Award, Rice Premedical Society, 1995  
Outstanding Associate, Lovett College, 1995,  
Cain Project Highlighted Teacher for Innovation 2003  
Outstanding Associate, Lovett College, 2004  
Distinguished Associate (Award for student advising and mentoring) 2002  
Distinguished Associate (Award for student advising and mentoring) 2003  
Hershel M. Rich Invention Award 2004  
Hershel M. Rich Invention Award, 2005  
Hamill Innovation Award, 2005  
Fellow American Academy of Microbiology (2006)  
E. Dell Butcher Professor of Biochemistry & Cell Biology

## **EDITORIAL BOARDS:**

Editorial Board, *Anaerobe*, 1994-2005

Editorial Board, *Applied Microbiology and Biotechnology*, 2002-2006

Editorial Board, *Electronic Journal of Biotechnology*, 1998-2006

## **SERVICE**

### **A. University Service**

#### **1. University Activities**

Member, Campus Safety Committee, 1984-1989

Member, Campus Safety Officer Recruiting Committee, 1989-1991

Member, Environmental Health and Safety Working Group, 1991-1993

Member, Strategic Planning Committee, Wiess School of Natural Sciences,  
Undergraduate Studies, 1996-1997

Member, Strategic Planning Committee for the Rice School, 1996-1997

Member, Wiess Scholars Program, 1994-1997

Member, McNair Scholars Program, 1998

Member, BioEngineering Program, 1994-1998

Member, Task Force on Graduate Student Teaching, 1999

Member, Admissions Committee, 1993-1996, 1998-2005

Member, Advisory Committee on Academic Advising, 1999-2000

Member, University Parking Study Advisory Committee, 1999-2000

Co-Chair, Admissions Committee, 2000-2001

Natural Sciences Advisor 1988-2005

Member, Financial Aid Appeal Committee, 2002-2005

Member, Athletic Admission Committee, 2002-2005

Faculty Mentor, Century Scholars Program, 2003-2005

Member, Training Committee NSF IGERT Program, 2001-2005

Member, Natural Sciences Faculty K-12 Resources Committee, 2004-2005

Member, Institute of Biosciences & Bioengineering Steering Committee, 2003-

Member, NIH Biotechnology Training Grant Steering Committee, 2004-

### **B. Professional Service**

Member, Alcohol Fuels Review Panel, U.S. Department of Agriculture, 1989-1991

Panelist, National Science Foundation Biotechnology, 1987, 1992-1993

Panelist, Life in Extreme Environments, NSF Interagency Grant Panel (LEn), 1998

Panelist, DOE Energy Biosciences

Organizing Committee for International Clostridium 2000 Meeting, Urbana, Illinois,  
1999-2000

Organizing Committee for International Clostridium 1994 Meeting, Northwestern  
University, 1994

Organizer 9<sup>th</sup> International Clostridium Meeting, Rice University, 2006

Institutional Review Board, The Methodist Hospital Research Institute 2005-

Member, Preceptor Committee, 1 Fellow, M.D. Anderson Cancer Institute, 2000-2002

Member, American Society for Microbiology, 1983-2004

Member, American Chemical Society, 1983-2004  
 Member, American Society for Biochemistry and Molecular Biology, 1983-2004  
 Member, American Association for the Advancement of Science, 1992-2004  
 Member, Society for Industrial Microbiology, 1995-2004 (Symposium session co-organizer, 1994-1996)  
 Invited Participant, Stanford University Microbial Genome Initiative, 1994-1996  
 Invited Panel Participant, Biofuels, Panel, USDA, 1994-1996  
 Reviewer, The Consortium for Plant-Biotechnology Research grants, 1998-1999, 2003-2005  
 Reviewer, Department of Energy grants, 1992-2000, 2005-6  
 Reviewer, U.S. Department of Agriculture grants, 1992-2004  
 Reviewer, Army Research grants, 2001-2006  
 Reviewer, National Science Foundation grants, 1992-1999, 2001-2006  
 Reviewer, Applied Microbiology and Biotechnology, Journal of Clinical Microbiology, Applied and Environmental Microbiology, Biotechnology and Applied Biochemistry, Metabolic Engineering, Chemosphere, Biochemistry, Environmental Science & Technology, Journal of Bacteriology, Nature Reviews Microbiology, Biotechnology & Bioengineering, Process Biochemistry, BioMed Central Microbiology, FEMS Microbiology Letters, Biotechnology Progress, Journal of Industrial Microbiology & Biotechnology, Molecular Microbiology, Nucleic Acid Research (in 2004-05 period)  
 Member, Graduate Ph.D. Thesis Committee, U.T. Medical School, Houston, 1993-1996  
 Member, Graduate Ph.D. Thesis Committee, St. John's University, 1993-1997  
 Outside Thesis Reviewer, University of Capetown, South Africa, 1998  
 External Advisory Committee for PPG, Stanford University -- Martin Brown "Development of New Hypoxic Cytotoxins for Cancer Therapy", 2003-2005  
 Member, BioHouston Genomics Task Force, 2004-05  
 Hosting researchers displaced by Allison (2001) and Katrina (2005)  
 Judge, Houston Science & Engineering Fair (usually team leader), 1991-2004  
 Judge, Odyssey of the Mind, Houston and State Competitions, 2001-2005  
 Collaborations with: K.-Y. San -- Department of Bioengineering, Rice University; N. Mantzaris -- Department of Chemical Engineering, Rice University; K. Zygorakis -- Department of Chemical Engineering, Rice University; Praveen V. Vadlani -- AgRenew, Inc; Joseph B. Hughes -- Department of Civil and Environmental Engineering, Georgia Tech; Farrukh Ahmad -- Groundwater Services, Inc.; Carl C. Zhang -- School of Natural and Applied Sciences, University of Houston, Clear Lake; S. Cox -- Department of Computational and Applied Mathematics, Rice University; Martin Brown -- Stanford; Yun Oh -- MD Anderson Cancer Center, Houston; E. T. Papoutsakis -- Northwestern

## **GRADUATE STUDENT SUPERVISION:**

### **Current Graduate Students:**

<b>Name:</b>	<b>Degree Sought:</b>
Sullivan, Leighann	Ph.D.

### **Past Graduate Students:**

Elizabeth A. Auger, Ph.D., "Studies on the Effects of Temperature, pH, and Anaerobiosis on Gene Expression in *E. coli* K-12," 1988, currently Faculty, Department of Biology, St. Joseph's College, Standish, Maine

Ed Belouski, M.A., "Cloning and Sequencing of Genes Involved in Glycolysis from *Clostridium acetobutylicum*," 1996 currently, DNA testing, Texas State Fish & Game Department, Palacios, Texas

Richard B. Gayle, III, Ph.D., "Construction and Characterization of *Escherichia coli* Plasmids Useful in the Manipulation of DNA," 1984, currently Research Scientist, Immunex Corp., Seattle, Washington

George L. Herrin, Jr., Ph.D., "The Effects of Altered Supercoiling on Expression from Bacterial Promoters," 1985, currently Director, State DNA Analysis Laboratory, Atlanta, Georgia

Shi-Yuan Meng, Ph.D., "Studies on the *cad* Operon of *Escherichia coli* K-12: A pH Regulatory System in Bacteria," 1992, currently Research Scientist, Amgen Inc., Thousand Oaks, California

Daniel J. Petersen, Ph.D., "Characterization of the Acetone Production Pathway Genes from *Clostridium acetobutylicum* ATCC 824," 1991, currently CODIS Administrator, Oregon State Police Crime Laboratory, Portland OR

David R. Russell, Ph.D., "Construction and Analysis of *Escherichia coli* Hybrid and Variant Promoters," 1983, currently Director of Plant Molecular Biology, Renessen, Chicago IL

Miles Scotcher, Ph.D., "Genetic Factors Affecting the Regulation of Solventogenesis in *Clostridium acetobutylicum* ATCC 824," 2004, currently Postdoctoral Fellow, University of Washington, Seattle

Xiao-Lu Shi, Ph.D., "Studies on the Regulation of Biodegradative Arginine and Lysine Decarboxylase Gene Expression in *Escherichia coli*," 1995, currently in Shanghai, China after Rockefeller and UT-Southwestern.

Tara Soughers, M.A., "Study of the Effect of DNA Secondary Structure on Reactivity of Hedamycin and Analysis of Systems for Directed Mutagenesis," 1985, currently in the Ministry in Moravia, New York

Polly S. Vermersch (Ledvina), Ph.D., "Genetic Strategies for Analyzing Proteins: Applications Utilizing the R388 Type II Dehydrofolate Reductase," 1988, currently Architect in private practice, Houston, Texas

Stephanie Wardwell, Ph.D., "Acetoin in *Clostridium acetobutylicum* ATCC 824," 1999, currently Patent agent, Washington, D.C.

John Wong, Ph.D., "Genetic Regulation in *Clostridium acetobutylicum* ATCC 824," 1995, Postdoctoral Fellow, Oregon Health Science Center, Portland

#### **POSTDOCTORAL SUPERVISION:**

<b>Name:</b>	<b>Year(s) Supervised:</b>
M. Ali	2001 – 2004
J. Cary	1987-1989
E. Green	1993-1997
G. Herrin	1985
K. Huang	1998 – 1999
S. Huang	1998
R. Kutty	2001–present
B. Lu	1998-1999
M. Lyrstis	1997–1999
P. D. Miller	1981-1984

M. Peredelchuk	1995-1998
R. Padda	1998-2000
C. Sass	1990-1991
K. Stim	1989-1995
M. Tyurin	1998-1999
Y-T. Yang	1999-2001
K. Yesland	1996-1997
J. Wong	1995
Y. Zhao	2000- 2003
Sagit Shalel-Levanon	2003 -2005
Y. C. Park	2004-2006
T. B. Causey	2004-2006

#### **VISITING FACULTY (SABBATICAL) SUPERVISION:**

**P. Lindahl** 1996

**H.Y. Song** 2002-2003

#### **PUBLICATIONS:**

1. Bennett, G.N., Mackey, J.K., Weibers, J.L., and Gilham, P.T., 2'-O-( $\alpha$ -methoxyethyl)-nucleoside 5'-diphosphates as "single-addition" substrates in the synthesis of specific oligoribonucleotides with polynucleotide phosphorylase, *Biochemistry*, **12**, 3956-3962 (1973).
2. Sninsky, J.J., Bennett, G.N., and Gilham, P.T., "Single-addition" and "transnucleotidation" reactions catalyzed by polynucleotide phosphorylase. Effect of enzymatic removal of inorganic phosphate during reaction, *Nucleic Acids Research*, **1**, 1665-1674 (1974).
3. Bennett, G.N., The use of the methoxyethyl blocking group in the enzymatic synthesis of specific oligonucleotides and in the chemical synthesis of guanosine tetraphosphate, Ph.D. Thesis, Purdue University (1974).
4. Bennett, G.N., and Gilham, P.T., "Single-addition" substrates for the synthesis of specific oligoribonucleotides with polynucleotide phosphorylase. Synthesis of 2'-O-( $\alpha$ -methoxyethyl)-nucleoside 5'-diphosphates, *Biochemistry*, **14**, 3152-3158 (1975).
5. Sninsky, J.J., Hawley, D.M., and Bennett, G.N., Modifications in the sugar moieties of nucleoside diphosphates that result in limited additions to oligonucleotide primers in polynucleotide phosphorylase reactions, *Fed. Proc.*, **34**, 702 (Abstract No. 2742) (1975).
6. Yanofsky, C., Korn, L., Lee, F., Bertrand, K., Bennett, G., and Schweingruber, M., The two transcription control sites in the tryptophan operon of *E. coli*, *Fed. Proc.*, **35**, 1343 (1976).
7. Bennett, G.N., Schweingruber, M.E., Brown, K.D., Squires, C., and Yanofsky, C., Nucleotide sequence of region preceding *trp* mRNA initiation site and its role in promoter and operator function, *Proceedings of the National Academy of Sciences U.S.A.*, **73**, 2351-2355 (1976).
8. Bennett, G.N., Gough, G.R., and Gilham, P.T., Guanosine tetraphosphate and its analogs. Chemical synthesis of guanosine 3',5'-di(pyrophosphate), deoxyguanosine 3',5'-di(pyrophosphate), guanosine 2',5'-bis(methylenediphosphonate), and guanosine 3',5'-bis(methylenediphosphonate), *Biochemistry*, **15**, 4623-4628 (1976).
9. Bennett, G.N., Brown, K.D., and Yanofsky, C., Nucleotide sequence of the promoter-operator region of the tryptophan operon from *E. coli* and *S. typhimurium*, *Fed. Proc.*, **36**, 878 (1977).
10. Franklin, N., and Bennett, G.N., DNA sequencing of the N gene of bacteriophage lambda, *Abstracts, Cold Spring Harbor Meeting on Bacteriophage and Single-Stranded DNA Phage*, **39** (1977).

11. Hawley, D.M., Sninsky, J.J., Bennett, G.N., and Gilham, P.T., Activity of polynucleotide phosphorylase with nucleoside diphosphates containing sugar ring modifications, *Biochemistry*, **17**, 2082-2086 (1978).
12. Bennett, G.N., Schweingruber, M.E., Brown, K.D., Squires, C., and Yanofsky, D., Nucleotide sequences of the promoter-operator region of the tryptophan operon of *Escherichia coli*, *J. Mol. Biol.* **121**, 113-137 (1978).
13. Bennett, G.N., Brown, K.D., and Yanofsky, C., Nucleotide sequence of the promoter-operator region of the tryptophan operon of *Salmonella typhimurium*, *J. Mol. Biol.*, **121**, 139-152 (1978).
14. Brown, K.D., Bennett, G.N., Lee, F., Schweingruber, M.E., and Yanofsky, C., RNA polymerase interaction at the promoter-operator region of the tryptophan operon of *Escherichia coli* and *Salmonella typhimurium*, *J. Mol. Biol.*, **121**, 153-177 (1978).
15. Bennett, G.N., and Yanofsky, C., Sequence analysis of operator constitutive mutants of the tryptophan operon of *Escherichia coli*, *J. Mol. Biol.*, **121**, 179-192 (1978).
16. Lee, F., Bertrand, K., Bennett, G., and Yanofsky, C., Comparison of the nucleotide sequences of the initial transcribed regions of the tryptophan operons of *Escherichia coli* and *Salmonella typhimurium*, *J. Mol. Biol.*, **121**, 193-217 (1978).
17. Stauffer, G.V., Zurawski, G., and Bennett, G.N., *In vivo* cloning of DNA regions carrying mutations linked to selectable genes: Application to mutations in the regulatory region of the *Escherichia coli* tryptophan operon, *Plasmid*, **2**, 498-502 (1979).
18. Franklin, N.C., and Bennett, G.N., The N-protein of bacteriophage lambda, defined by its DNA sequence, is highly basic, *Gene*, **8**, 107-119 (1979).
19. Manly, S.P., and Bennett, G.N., Effects of the tryptic core of *lac* repressor on the methylation pattern of the operator DNA sequence, *Fed. Proc.*, **39**, 1607 (1980).
20. Nichols, B.P., Miozzari, G.F., van Cleemput, M., Bennett, G.N., and Yanofsky, C., Nucleotide sequences of the *trp* G regions of *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium* and *Serratia marcescens*, *J. Mol. Biol.*, **142**, 503-517 (1980).
21. Oppenheim, D.S., Bennett, G.N., and Yanofsky, C., *Escherichia coli* RNA polymerase and *trp* repressor interaction with the promoter-operator region of the tryptophan operon of *Salmonella typhimurium*, *J. Mol. Biol.*, **144**, 133-142 (1980).
22. Sumner, W., II, and Bennett, G.N., Anthramycin inhibition of restriction endonuclease cleavage and its use as a reversible blocking agent in DNA constructions, *Nucleic Acids Research*, **9**, 2105-2119 (1981).
23. Russell, D.R., and Bennett, G.N., Characterization of the  $\beta$ -lactamase promoter of pBR322, *Nucleic Acids Research*, **9**, 2517-2533 (1981).
24. Russell, D.R., and Bennett, G.N., Cloning of small DNA fragments containing the *Escherichia coli* tryptophan operon promoter and operator, *Gene*, **17**, 9-18 (1982).
25. Russell, D.R., and Bennett, G.N., Analysis of *in vitro* constructed *E. coli* promoters, *Fed. Proc.*, **41**, 758 (1982).
26. Herrin, G.L., Jr., Russell, D.R., and Bennett, G.N., A stable derivative of pBR322 conferring increased tetracycline resistance and increased sensitivity to fusaric acid, *Plasmid*, **7**, 290-293 (1982).
27. Bennett, G.N., Formation of alkali labile linkages in DNA by hedamycin and use of hedamycin as a probe of protein-DNA complexes, *Nucleic Acids Research*, **10**, 4581-4594 (1982).
28. Russell, D.R., and Bennett, G.N., Construction and analysis of *in vivo* activity of *E. coli* promoter hybrids and promoter mutants that alter the -35 to -10 spacing, *Gene*, **20**, 231-243 (1982).
29. Herrin, G.L., Jr., and Bennett, G.N., The effect of supercoiling on expression from a series of bacterial fusion promoters, *Fed. Proc.*, **42**, 2262 (1983).
30. Manly, S.P., Bennett, G.N., and Matthews, K.S., Perturbation of *lac* operator DNA modification by tryptic core protein from lactose repressor, *Proceedings of the National Academy of Sciences U.S.A.*, **80**, 6219-6223 (1983).



31. Russell, D.R., Auger, E.A., Vermersch, P.S., and Bennett, G.N., Role of DNA regions flanking the tryptophan promoter of *Escherichia coli*. I. Insertion of synthetic oligonucleotides, *Gene*, **32**, 337-348 (1984).
32. Herrin, G.L., Jr., and Bennett, G.N., Role of DNA regions flanking the tryptophan promoter of *Escherichia coli*. II. Insertion of *lac* operator fragments, *Gene*, **32**, 349-356 (1984).
33. Manly, S.P., Bennett, G.N., and Matthews, K.S., Enzymatic digestion of operator DNA in the presence of the *lac* repressor tryptic core, *J. Mol. Biol.*, **179**, 335-350 (1984).
34. Russell, D.R., Miller, P.D., and Bennett, G.N., *In vitro* characterization of hybrid promoters and altered tryptophan operon promoters, *Biochemistry*, **24**, 1410-1417 (1985).
35. Gayle, R.B., III, Vermersch, P.S., and Bennett, G.N., Construction and characterization of pBR322-derived plasmids with deletions of the RNA I region, *Gene*, **41**, 281-288 (1986).
36. Vermersch, P.S., Klass, M.R., and Bennett, G.N., Use of bacterial DHFR-II fusion proteins to elicit specific antibodies, *Gene*, **41**, 289-297 (1986).
37. Herrin, G.L., Jr., and Bennett, G.N., The effects of nalidixic acid on expression from related *E. coli* promoters, *Biochem. Biophys. Res. Commun.*, **135**, 411-418 (1986).
38. Auger, E.A., and Bennett, G.N., Temperature optimization of *in vivo* expression from the *E. coli trp* and *trp::lac* promoters, *Biotechnology Letters*, **9**, 157-162 (1987).
39. Gayle, R.B., III, Auger, E.A., Gough, G.R., Gilham, P.T., and Bennett, G.N., Formation of *Mbo*II vectors and cassettes using asymmetric *Mbo*II linkers, *Gene*, **54**, 221-228 (1987).
40. Vermersch, P.S., and Bennett, G.N., The use of a selectable *Fok*I cassette in DNA replacement mutagenesis of the R388 dihydrofolate reductase gene, *Gene*, **54**, 229-238 (1987).
41. Vermersch, P.S., and Bennett, G.N., Synthesis and expression of a gene for a mini Type II dihydrofolate reductase, *DNA*, **7**, 243-251 (1988).
42. Cary, J.W., Petersen, D.J., Papoutsakis, E.T., and Bennett, G.N., Cloning and expression of *Clostridium acetobutylicum* phosphotransbutyrylase and butyrate kinase genes in *Escherichia coli*, *J. Bacteriol.*, **170**, 4613-4618 (1988).
43. Auger, E.A., and Bennett, G.N., Regulation of lysine decarboxylase activity in *Escherichia coli* K-12, *Arch. Microbiol.*, **15**, 466-468 (1989).
44. Auger, E.A., Redding, K.E., Plumb, T., Childs, L.C., Meng, S.-Y., and Bennett, G.N., Construction of *lac* fusions to the inducible arginine- and lysine decarboxylase genes of *Escherichia coli* K12, *Molecular Microbiol.*, **3**, 609-620 (1989).
45. Clark, S.W., Bennett, G.N., and Rudolph, F.B., Isolation and characterization of mutants of *Clostridium acetobutylicum* ATCC 824 deficient in acetoacetyl-Coenzyme A:acetate/butyrate:Coenzyme A transferase (EC 2.8.3.9) and in other solvent pathway enzymes, *App. Environ. Microb.*, **55**, 970-976 (1989).
46. Cary, J.W., Petersen, D.J., Bennett, G.N., and Papoutsakis, E.T., Methods for cloning key primary metabolic enzymes and ancillary proteins associated with the acetone-butanol fermentation of *Clostridium acetobutylicum*, *Ann. N.Y. Acad. Sci.*, **589**, 67-81 (1990).
47. Brito, R.M.M., Reddick, R., Bennett, G.N., Rudolph, F.B., and Rosevear, P.R., Characterization and stereochemistry of cofactor oxidation by a type II dihydrofolate reductase, *Biochemistry*, **29**, 9825-9831 (1990).
48. Cary, J.W., Petersen, D.J., Papoutsakis, E.T., and Bennett, G.N., Cloning and expression of *Clostridium acetobutylicum* ATCC 824 acetoacetyl-Coenzyme A:acetate/butyrate:Coenzyme A-transferase in *Escherichia coli*, *App. Environ. Microb.*, **56**, 1576-1583 (1990).
49. Petersen, D.J., and Bennett, G.N., Purification of acetoacetate decarboxylase from *Clostridium acetobutylicum* ATCC 824 and cloning of the acetoacetate decarboxylase gene in *Escherichia coli*, *App. Environ. Microb.*, **56**, 3491-3498 (1990).
50. Wang-Bennett, L.T., Liebl, D.J., and Bennett, G.N., Targeted neuronal lesion induced by photosensitizing dyes, *Brain Research*, **534**, 122-128 (1990).

51. Petersen, D.J., Welch, R.W., Rudolph, F.B., and Bennett, G.N., Molecular cloning of an alcohol (butanol) dehydrogenase gene cluster from *Clostridium acetobutylicum* ATCC 824, *J. Bacteriol.*, **173**, 1831-1834 (1991).
52. Vermersch, P.S., and Bennett, G.N., Stability of mutant Type II dihydrofolate reductase proteins in suppressor strains, *J. Biotechnology*, **19**, 49-66 (1991).
53. Hassani, M., Saluta, M.V., Bennett, G.N., and Hirshfield, I.N., Partial characterization of a *lysU* mutant of *Escherichia coli* K-12, *J. Bacteriol.*, **173**, 1965-1970 (1991).
54. Petersen, D.J., and Bennett, G.N., Cloning of the *Clostridium acetobutylicum* ATCC 824 acetyl coenzyme A acetyltransferase (Thiolase; EC 2.3.1.9), *Appl. Environ. Microbiol.*, **57**, 2735-2741 (1991).
55. Petersen, D.J., and Bennett, G.N., Enzymatic characterization of a nonmotile, nonsolventogenic *Clostridium acetobutylicum* ATCC 824 mutant, *Current Microbiol.*, **23**, 253-258 (1991).
56. Petersen, D.J., Welch, R.W., Walter, K.A., Mermelstein, L.D., Papoutsakis, E.T., Rudolph, F.B., and Bennett, G.N., Cloning of an NADH-dependent butanol dehydrogenase gene from *Clostridium acetobutylicum*, *Ann. N.Y. Acad. Sci.*, **646**, 94-98 (1991).
57. Meng, S.-Y., and Bennett, G.N., Nucleotide sequence of the *Escherichia coli cad* operon: A system for detoxification of low extracellular pH, *J. Bacteriol.*, **174**, 2659-2669 (1992).
58. Meng, S.-Y., and Bennett, G.N., Regulation of the *Escherichia coli cad* operon: Location of a site required for acid induction, *J. Bacteriol.*, **174**, 2670-2678 (1992).
59. Mermelstein, L.D., Welker, N.E., Bennett, G.N., and Papoutsakis, E.T., Expression of cloned homologous fermentative genes in *Clostridium acetobutylicum* ATCC 824, *Bio/Technology*, **10**, 190-195 (1992).
60. Lee, S.Y., Bennett, G.N., and Papoutsakis, E.T., Construction of *Escherichia coli*-*Clostridium acetobutylicum* shuttle vectors and transformation of *Clostridium acetobutylicum* strains, *Biotechnology Letters*, **14**, 427-432 (1992).
61. Tolentino, G.J., Meng, S.Y., Bennett, G.N., and San, K.-Y., A pH-regulated promoter for the expression of recombinant proteins in *Escherichia coli*, *Biotechnology Letters*, **14**, 157-162 (1992).
62. Welch, R.W., Clark, S.W., Bennett, G.N., and Rudolph, F.B., Effects of rifampicin and chloramphenicol on product and enzyme levels of the acid- and solvent-producing pathways of *Clostridium acetobutylicum* (ATCC 824), *Enzyme Microbiol. Technol.*, **14**, 277-283 (1992).
63. Hassani, M., Pincus, D.H., Bennett, G.N., and Hirshfield, I.N., Temperature-dependent induction on an acid-inducible stimulon of *Escherichia coli* in broth, *App. Environ. Microbiol.*, **58**, 2704-2707 (1992).
64. Lee, S.Y., Mermelstein, L.D., Bennett, G.N., and Papoutsakis, E.T., Vector construction, transformation, and gene amplification in *Clostridium acetobutylicum* ATCC 824, *Biochem. Eng.*, **VII**, *Ann. New York Acad. Sci.*, **665**, 39-51 (1992).
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5. A New Technique to Increase the Intracellular Levels of CoA and Acetyl-CoA in *Escherichia coli* with the Goal of Enhancing Production of Compounds that Require Acetyl-CoA in their Biosynthesis (filed 2003) with R.V. Vadali and K.-Y. San.
6. A New Technique to to Manipulate *Escherichia coli* Metabolic Pathways to Increase Flux through the Intracellular Acetyl-CoA Node and Divert this Flux to Enhance Productivities of Compounds that Require Acetyl-CoA in their Biosynthesis (filed 2003) with R.V. Vadali and K.-Y. San.

7. A Novel Approach to Construct High Molar Succinate Yield Production Strains by Increasing the Intracellular NADH Availability in *Escherichia coli* (filed 2003) with A. Sanchez and K.-Y. San.
8. Aerobic Production of Succinate (filed 2003) with H. Lin and K.-Y. San.
9. Increased Bacterial Acetyl-CoA Pool (filed March 24, 2003) with K.-Y. San and R.V. Vadali.
10. Increasing Intracellular NADPH Availability in *E. coli* (filed 2003) with A. Sanchez and K.-Y. San.
11. Simultaneous Anaerobic Production Of Isoamyl Acetate And Succinic Acid (12/22/04, 12/22/05) 31175413-015001, Ka-Yiu San, Cheryl Dittrich, Ailen Sanchez and George N. Bennett, patent pending
12. High Succinate Producing Bacteria (9/17/04, 9/17/05) 31175413-014001, Ka-Yiu San Henry Lin, Ailen Sanchez and George N. Bennett, patent pending
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16. “Novel PEPC to Produce Succinate” George N. Bennett, Ka-Yiu San; Mary Lou Harrison
17. “UBICA IN *E. Coli*” Ka-Yiu San and George N. Bennett